SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzyme Assay of ARYLAMINE ACETYLTRANSFERASE
(EC 2.3.1.5)

PRINCIPLE:

Acetyl-CoA + Dye $\xrightarrow{AAT}$ > CoASH + Acetylated Dye

Abbreviations used:
Acetyl-CoA = Acetyl Coenzyme A
Dye = p-Nitroaniline
AAT = Arylamine Acetyltransferase
CoASH = Coenzyme A

CONDITIONS:

T = 25°C, pH = 8.0, A_{400nm}, Light path = 1 cm

METHODS: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 8.0 at 25°C with 1 M KOH.)

B. 100 mM β-Mercaptoethanol Solution (β-ME)
(Prepare 5 ml in deionized water using 2-Mercaptoethanol, Sigma Prod. No. M-6250.)

C. 30 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 5 ml in deionized water using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS.)

D. 1.5 mM p-Nitroaniline Solution (Dye)
(Prepare 5 ml in deionized water using p-Nitroaniline, Sigma Prod. No. N-2128.)

E. 6.0 mM Acetyl Coenzyme A Solution (Acetyl CoA)
(Prepare 1 ml in deionized water using Acetyl Coenzyme A (C2:0), Sodium Salt, Sigma Prod. No. A-2056. **PREPARE FRESH**.)

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REAGENTS: (continued)

F. Arylamine Acetyltransferase Enzyme Solution
(Immediately before use, prepare a solution containing 100 mg/ml of Arylamine Acetyltransferase in cold Reagent A.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	Test	Blank
Reagent A (Buffer)	1.50	1.50
Reagent B (β-ME)	0.15	0.15
Reagent C (EDTA)	0.10	0.10
Reagent D (Dye)	0.20	0.20
Reagent F (Enzyme Solution)	1.00	1.00

Mix by inversion and equilibrate to 25°C. Monitor the A_{400nm} until constant (approx. 4 minutes) using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Acetyl CoA)	0.05	------
Deionized Water	------	0.05

Immediately mix by inversion and record the increase in A_{400nm} for approximately 5 minutes. Obtain the ΔA_{400nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{400nm}\ \text{Test} - \Delta A_{400nm}\ \text{Blank})(1000)(3)(df)}{(11.19)(1)}$$

1000 = Conversion from µmoles to nanomoles
3 = Volume (in milliliters) of assay
df = Dilution factor
11.19 = Millimolar extinction coefficient of acetylated dye
1 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

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CALCULATIONS: (continued)

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will acetylate 1.0 nanomole of p-nitroaniline per minute at pH 8 at 25°C.

FINAL CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 83 mM potassium phosphate, 5 mM β-mercaptoethanol, 1 mM ethylenediaminetetraacetic acid, 0.1 mM p-nitroaniline, 0.1 mM acetyl coenzyme A and 100 mg of arylamine acetyltransferase.

REAGENTS:

Brooks, S.P.J. and Storey, K.B., (1993) *Analytical Biochemistry* **212**, 452-456

NOTES:

1. This assay is based on the cited reference.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.